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BIOSIMILARS: A BOON TO INFERTILE WOMEN

Ashwati V. Nair[1]*, Purnima D. Hamrapurkar[2] and Rajani B. Athawale[3]

[1]Master's in Pharmaceutical Analysis, Research Student of the Department of Pharmaceutical Analysis. Principal K.M. Kundnani College of Pharmacy, plot No.23, Jote Joy building, Rambhau Salgaonkar Marg, Cuffe Parade, Mumbai 400005, Maharashtra, India.
[2]Ph.D. in Analytical Chemistry, Professor and HOD of Department of Pharmaceutical Analysis. Principal K.M. Kundnani College of Pharmacy, plot No.23, Jote Joy building, Rambhau Salgaonkar Marg, Cuffe Parade, Mumbai 400005, Maharashtra, India.
[3]Ph.D. in Pharmaceutics, Professor and HOD of Department of Pharmaceutics. Principal K.M. Kundnani College of Pharmacy, plot No.23, Jote Joy building, Rambhau Salgaonkar Marg, Cuffe Parade, Mumbai 400005, Maharashtra, India.

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***Corresponding Author**
Ashwati V. Nair
Master's in Pharmaceutical Analysis, Research Student of the Department of Pharmaceutical Analysis. Principal K.M. Kundnani College of Pharmacy, plot No.23, Jote Joy building, Rambhau Salgaonkar Marg, Cuffe Parade, Mumbai 400005, Maharashtra, India.

ABSTRACT

Giving birth to a child is every human's desire and nowadays the rate of infertility is increasing due to many factors. Therefore there are various advances in the medical field to treat infertile women; one amongst them is by using biologics and biosimilars. The recombinant human follicle-stimulating hormone (r-hFSH); a biotechnologically derived hormone developed as an original biological molecule, is proven to be advantageous in the induction of ovulation, and thus formulated as biosimilars. Biosimilars are proven to be similar to original biologics in terms of structure, safety, efficacy, biological activity, and immunogenicity. The ultimate aim of the manufacturing and the entire developmental steps of biosimilar products focuses to match its profile with the original biologic and to accomplish this aim various comparability testings are carried out like physicochemical and functional groups, non-clinical, clinical, and confirmatory. Thus increasing another treatment option to the infertile female population to attain fertility.

KEYWORDS: Infertility, Biosimilars, recombinant human follicle-stimulating hormone, manufacture, developmental steps.

1. INTRODUCTION

1.1 Infertility

Giving birth to a child and raising are significant in every human's life and are linked with feelings of happiness, togetherness, and completeness. Nowadays, fertility rates are declining. Infertility affects an estimated 15% of couples, accounting for 48.5 million couples globally.[1] National Institute for Health and Clinical Excellence (NICE 2013), projected that infertility ought to be outlined as a failure to conceive once regular unprotected sexual intercourse for two years within the absence of known reproductive pathology.[2,3] In line with World Health Organisation; it says that infertility is 'a disease of the reproductive system outlined by the failure to attain a clinical gestation once twelve months or more of regular unprotected sexual activity'.[3] Infertility is the inability of a couple to attain pregnancy over one year in a woman under 35 years of age or 6 months in a woman above 35 years of age despite having regular unprotected sexual intercourse.[4]

1.2 Causes of infertility

The most common causes of infertility in women are ovarian dysfunction with anovulation (25–35%),[5,6] fallopian tube dysfunction (25-30%),[7] the disorder of menstruation (20%),[7] pathologies of oviducts (20–25%),[5,6] endometriosis (10–20%),[5,6] uterine pathology (fibroids, adhesions 5–10%)[5,6] and unexplained infertility (20–30%).[5,6] Lifestyle and environmental factors leading to psychological disorders also contribute to infertility. Stress is the most common cause. Experimental data suggest chronic or severe stress leads to anovulation and amenorrhea in women.[8,9] Smoking and alcohol consumption are yet another concerning lifestyle factors that decrease fertility rates. The decline in the world fertility rate from 1990 to 2018 is depicted in Fig 1 which indicates the decline in the fertility rate worldwide in every consecutive year.

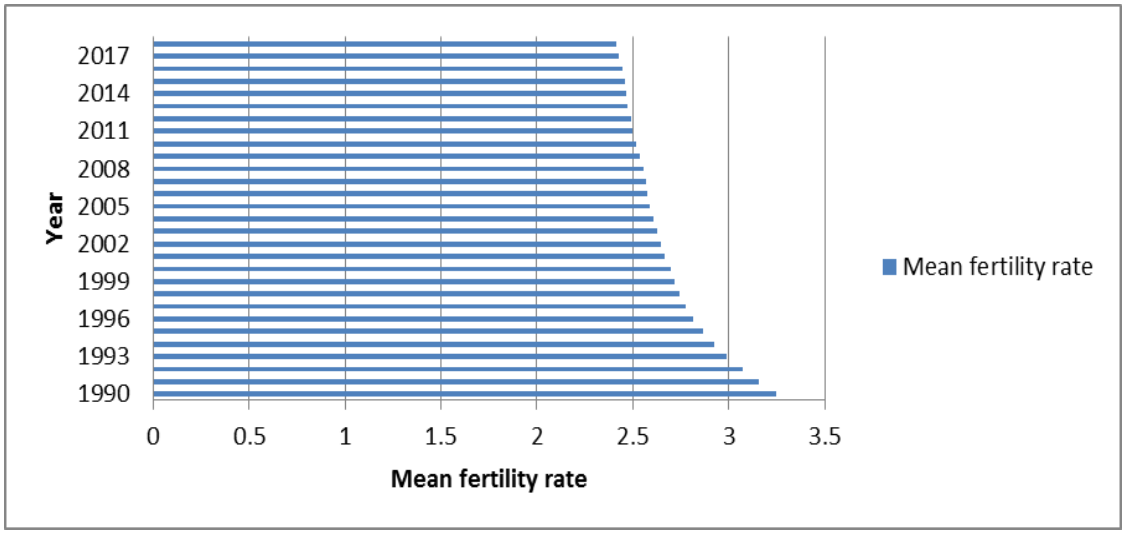

Fig. 1: World mean fertility rate, 1990-2018.[10]

2. Follicle-stimulaing hormone (fsh)

2.1 Significance

Various hormones play a significant role in fertilization. One of the major hormones is a follicle-stimulating hormone (FSH). It is a gonadotropin-releasing hormone (GnRH). It plays a significant role in growth, sexual development, and reproduction including follicular development and ovulation.[11] Inadequate concentrations of FSH caused either by deficient FSH synthesis or secretion, is characterized by abnormal or the absence of ovulation which significantly affects fertility. Administration of FSH has been used with success to treat fertility disorders mainly in ovulation induction because it has shown its efficiency in achieving ovulation in anovulatory infertile women, subfertility, and IVF (in-vitro fertilization).

2.2 Advantages of r-hFSH

Assisted reproductive technology (ART) is one such therapeutic application for recombinant proteins, and specifically for recombinant human follicle-stimulating hormone (rhFSH).[12] Proteins manufactured by recombinant techniques are regarded as safe, and eventually, could replace plasma-derived or urine-derived therapeutic proteins because of the customary of care.[13] Clinical pregnancy rates and safety – in terms of avoiding ovarian hyperstimulation syndrome – with rhFSH are the same as those achieved with purified urinary gonadotropins; however, the recombinant protein offers some significant advantages.[14] The development of recombinant DNA technology to supply recombinant human FSH (r-hFSH) has resulted in important enhancements within the standard and availableness of this product for clinical

use.[15] The main advantage could be a considerably lower number of vials/patients and therefore a slight (although non-significant) increase within the delivery rate with r-FSH, the cost-minimization analysis showed a 9.4% reduction within the overall medical aid value per born baby in favor of r-FSH.[15] Biosimilar FSH preparations have a comparatively high maternity rate (chemical and clinical) than HP (highly purified) urinary FSH.[16]

2.3 Structure of r-hFSH

The structure of r-hFSH consists of the following groups:

1. Amino acid residue sequence[17]

The complete amino acid residue sequences of r-hFSH α and β subunits as outlined by the automatic sequencing of peptides were obtained by digestion of enzyme trypsin of the isolated subunits. The sequence 18- and 22- peptide fragments are accustomed to derive the primary structure of the protein of the r-hFSH α and β subunits respectively.

2. Sites for glycosylation[17]

The glycosylated peptide from the r-hFSH α and β subunits that contain amino acid residue sequences determines the position of oligosaccharide chain attachments inside the primary subunits structure. Glycosylation at a particular location within the primary amino acid residues sequence from α and β subunits are Asn 52 and 78 on α subunit and Asn 7 and 24 on β subunits.

3. Composition of monosaccharides

The composition of monosaccharides of α and β subunits of r-hFSH contains mannose, fucose, N-acetyl-glucosamine, galactose, and sialic acid and is determined by high-performance anion-exchange chromatography.[17]

3. Original biological product

The original biological product is a biotechnologically derived patented product. Gonal-f®[18] is the original biologics for the treatment of infertility manufactured by Merck-Serono INC which contains Follitropin alfa.[18] It is formulated as an injection for subcutaneous administration.[18] The expiration of patent of the original biological product has created a replacement chance for patients to use an analogous effective product at a reduced price because it contains a similar biological entity (API) and shares similarity regarding quality, safety, and efficacy when compared with original biologics i.e. biosimilar products.[19]

4. Biosimilars

4.1 Overview

Biological products whose active ingredients or formulations are slightly changed to improve efficacy or enhance the therapeutic regimen are separately distinguished as "biobetters".[19] This product could endure favorable pharmacokinetic or pharmacodynamic alterations however the therapeutic attributes ought to be the same as that of the reference drug. In line with European Medicines Agency (EMA); a biosimilar is a biological medicine highly similar to original biological medicine already approved in the EU (called 'reference medicine') in terms of structure, biological activity, efficacy, safety, and immunogenicity profile.[20] Acknowledging biosimilarity maybe a result of a comparability exercise lasting from 6 to 12 years, that contains head-to-head quality, and preclinical and clinical studies, the ultimate goal of that is to exclude any relevant distinction between the biosimilar and therefore the reference medicinal product.[21] Possibilities of picking up minor molecular variations are exaggerated by using highly sensitive state-of-the-art physicochemical and biological activity analytical procedures whose sensitivity has exponentially exaggerated within the last ten years.[21] If the head-to-head quality comparability assessment doesn't reveal an important divergence between each biological medicines, it is uncertain that subsequent comparative Phase III trials will reveal any distinction within the light of the magnitude of the inter-patient variability.[22] Non-clinical and bioequivalence tests are essential. There are two Biosimilar products for infertility treatment. They are Ovaleap®[23] and Bemfola®[24] containing Follitropin alfa which is similar to the reference original biologics Gonal-f®.[18] Ovaleap® is the biosimilar product of Teva Pharmaceuticals[23] and Bemfola® of Finox Biotech AG.[24]

4.2 Development of biosimilars

The development of biosimilars follows a series of multi-steps which aims at matching with the original biomolecule.

Step 1: Definition of target

The attributes of the originator are the target for the development of biosimilars. So, their elaborated understanding of nature, variations, and clinical features ought to be best-known.

Definition of the target for every attribute is governed by two factors

i. The variability of the attribute within the originator may be shown by clinical and post-marketing surveillance i.e. making certain whether or not the product is safe and effective within the projected attributes. The structure of proteins is the sequence of

amino acids and thus the genes encoding for it is determined by the 3-D folding of the polypeptide chain. Furthermore, glycoproteins carry branched sugar residues at distinct amino acid side chains, that arise through post-translational modification within the endoplasmatic reticulum (N-glycosylation) and also the Golgi-apparatus (O-glycosylation)[25] where it may lead to many glycoforms of the same amino acid sequence. This would be caused either by inherent batch-to-batch variability within the manufacturing process or planned changes within the production process.[26] The variation in sugar component isn't problematic since it depends on the metabolic status of individuals. The fundamental blueprint is given by glycosylation enzymes in every cell line and most cases, a similar cell line is employed for biosimilars as for originators.[25] Changes within the production process need to be approved earlier and should befit to the stringent international guideline ICH Q5E,[28] which stipulates that changes haven't unfavorable impacts on the safety or efficacy of a drug.[27] Therefore, it's acceptable according to EMA quality guidelines to outline the complete variability of the originator as target: "The ranges are known before and when the observed shift in quality profile might unremarkably be used to support the biosimilar comparability exercise at the quality level, as either range is representative of the reference medicinal product wherein the quality attribute values that are beyond or within the range (s) determined for a quality attribute of the reference biological product should be appropriately reasoned concerning their potential impact on safety and efficacy".[28]

ii. The clinical relevance of the attributes which is of major importance and the parameters included are (a)pharmacokinetic studies where the critical attributes are for absorption, distribution, and metabolism, (b) safety associated with adverse events due to interaction with the target because of its high specificity, (c) immunogenicity where the critical attributes are amino acid sequence and structure, their aggregates, associated disulfide bridges, free thiols, possible degradation products, glycosylation products, etc., and (d) efficacy which implies binding to the target and effector functions.[25]

Step 2: Target-based development

The successful target-based development depends on cell lines and its manufacturing process which finally defines the target. From many clones produced in the cell lines, the one which closely resembles the originator molecule is selected and is cultivated in a bioreactor considering various attributes. The target in the downstream process is achieving maximum

purity. The target-based development is further divided into recombinant cell line development, upstream development, downstream (Purification) development, and drug product development.

Step 3: Similarity characterization of biosimilars

After target based development, the similarity is established and evaluated with the originator by the attributes to be matched like physical (primary structure, post-translational modifications), chemical (impurities), and biological activity.[29]

Step 4: Reduction in regulatory aspects

Functional and biological methods used for antibodies test all functions of the molecule individually and combined. They range from target binding to highly complex and sensitive measurement of the ADCC in a biological system and these are more sensitive than the results from toxicological investigations animals, for which reason EMA does not recommend animal testing in case of high similarity.[25]

Step 5: Confirmation by clinical trials

Various clinical trials are conducted to confirm the biosimilarity to detect the significant difference.[29]

4.3 Manufacturing processes

The characteristics of the final biosimilar products depend on the manufacturing process. A small change in the manufacturing process could lead to issues in protein stability and post-translational modifications mainly glycosylation since it has an impact on the biological activity which indeed affects the safety, efficacy, and immunogenicity. So, by taking into considerations all the critical processing parameters, the manufacturing must be carried out. The transfection of the pre-adapted host cell line dihydrofolate reductase deficient Chinese Hamster Ovary cells with the plasmids which code for the alfa and beta-chain (transfection plasmids attained from the vector pCLH3AXSV2DHFR and vector pCLH3AXSV2ODC holding the genomic DNA fragments enclosing the α and β human FSH genes respectively) [18] is performed under serum-free culture conditions. A two-tiered cell banking system of Master Cell Bank (MCB) and Working Cell Bank (WCB) was considered. MCB is used to express the desired therapeutic proteins of the drug substances and WCB for the selection of clone. The production cells from each vial of WCB are used for each production batch. The WCB is then thawed. The cultivation system for cell propagation and production consists of

two bioreactors, a first stirred tank bioreactor (up to 15 L) and a second perfusion bioreactor (up to 90 L) with ultrasonic cell retention system.[30] The fermentation is a two-step batch process that continued with continuous harvesting from the second bioreactor. The collection of supernatant is initiated after a cell density of at least 2 x 10^6 cells/ml is reached.[30] In every three-day intervals, harvesting is performed. Depth filter is used for clarification followed by 0.2 µm filtration. The end of production cells (EOPCs) also known as the sample of suspension of cells, is taken from the second bioreactor which operates in cell retention mode on the last day of production harvest to end the production cell bank. The downstream or purification process containing ultrafiltration, anion-exchange chromatography, ammonium sulfate precipitation and filtration, hydrophobic interaction chromatography followed by second ultrafiltration, hydroxyapatite chromatography, terminal nanofiltration finally sterile filtration and the purified active substance in 20mM sodium phosphate buffer pH 7.0 is stored under appropriate conditions until formulation into the finished product.[30]

4.4 Clinical particulars (Therapeutic Indications and its posology)

For women with Anovulation (including polycystic ovarian syndrome) who have become resistant to treatment with clomifene citrate,[31] a normally used regimen begins at 75-150 IU FSH daily and is increased preferably by 37.5 or 75 IU at 7 or ideally 14-day intervals if necessary, to get adequate, however not excessive, response. The maximal daily dose is usually not over 225 IU FSH.[32] For stimulation of multi follicular development in women experiencing superovulation for assisted reproductive technologies (ART) like in-vitro fertilization (IVF), gamete intrafallopian transfer and zygote intra-fallopian transfer,[31] a normally used regimen for superovulation involves the administration of 150-225 IU of follitropin alfa daily, which begins on days two or three of the cycle. Treatment is sustained until requisite follicular development has been attained.[32] For women containing anovulation due to severe Luiteneizing Hormone and Follicle-stimulating hormone deficiency (endogenous serum LH level < 1.2 IU/L),[31] a suggested regimen begins at 75 IU of lutropin alfa daily with 75-150 IU FSH. If an FSH dose increase is deemed acceptable, dose adjustment should preferably be after 7-14 day intervals and preferably by 37.5-75 IU increments. It may be acceptable to increase the period of stimulation in any one cycle to up to 5 weeks.[32]

5. Comparison of biosimilars with original biologic

5.1 Comparison of structural characteristics

The reduced and non-reduced molecular masses were determined by using SDS-PAGE and was found to be similar (The main band approximately 40 kDa for reduced and the main band approximately 20 kDa; additional faint bands between 16 and 20 kDa for non-reduced) while the native α and β subunit was found to be 14.6 kDa and 17.8/18.3 kDa respectively when analyzed by MALDI-TOF.[33] The primary structure and peptide mapping were performed using trypsin, endo Lys-C, and endo Glu-C then by ESI-MS for determination of peptides in which all the determined peptide masses correspond to the exactly expected masses whereas, for secondary structure- structural topology when analyzed by far-UV circular dichroism spectroscopy found that the samples are superimposable with the FSH reference standard indicating identical structural conformations and folding.[33] In the isoform distribution profile using isoelectric focusing, the pI values were found to be in the range of 3.6- 5.3 and 9 isoforms were detected.[33]

5.2 Comparison with clinical trials

a. Study population

The women whose ages are in between 18 and 38 years with the body mass index (BMI) between 18 and 30 kg/m^2 and have a regular menstrual cycle in 21 to 35 days are selected for clinical trials.[34] The major criteria considered are basal FSH levels should be less than 10 IU/L (cycle day 2-5), Estradiol levels should be <50 pg/ml (first day of FSH administration), and total antral follicle count should be 10-15 follicles.[35] The exclusion factors are women who had more than two unsuccessful IVF cycles or more than three miscarriages;[34] severe ovarian hyperstimulation syndrome (OHSS), severe endometriosis, polycystic ovaries, poor response to Gonadotropin treatment, and under any hormonal treatment in one month before the start of the treatment with an exception of levothyroxine treatment.[35]

b. Study design

On the 21st day, selected females were given gonadotropin-releasing hormone agonists to down-regulate endogenous FSH.[35] The confirmation of down-regulation was done with estradiol level <50 pg/ml, a shredded endometrium thickness of less than 5 mm, no ovarian cysts, and a negative pregnancy test, participants were randomized to receive a dose of 150 IU/L either from biosimilar products or Gonal-f®.[34] After 6 days of treatment with r-hFSH, in case of ovarian hyperstimulation (OHSS), the dose reduction is done. Women with at least

3 follicles of a mean diameter of 17mm and estradiol levels below 5500 pg/ml, then received a dose of 250 µg r-HCG (Ovitrelle, Merck Serono, Germany) to induce follicular maturation and trigger ovulation and after 34-37 hours, checked for oocyte retrieval. Embryo transfer takes place within 2-5 days after oocyte retrieval.[34] Evaluation of biochemical pregnancy and clinical pregnancy was done at 2 weeks and 5-6 weeks respectively.[36] Antibody detection was done after 12 weeks by using the immunoassay method to check for the presence of anti-FSH antibody.[36] The summary is shown in Fig. 2.

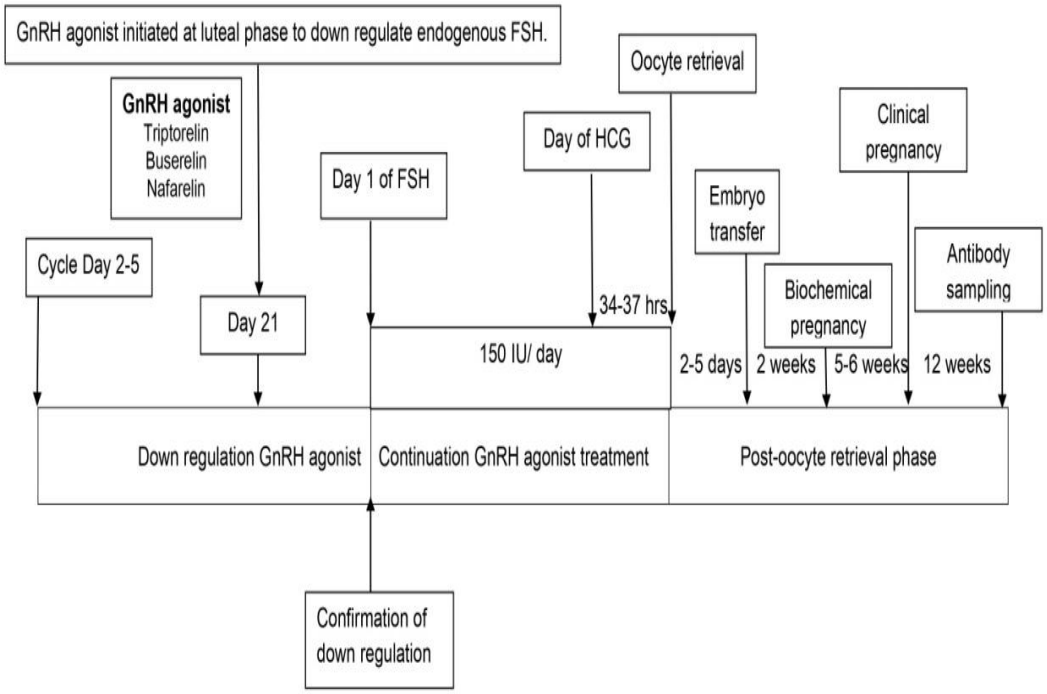

Fig. 2 Study design.

c. Disposition of patients

For studies using Bemfola® and Gonal-f® initial enrolment were 460 and for Ovaleap® and Gonal-f® were 398 out of which 88 and 99 failed respectively during screening due to various reasons. So the total number of patients who were intended to treat was 372 and 299 respectively. Amongst 372 patients, 249 patients have tested with Bemfola® and 129 patients with Gonal-f® while amongst 299 patients, 153 patients have tested with Ovaleap® and 146 with Gonal-f®. In the randomized treatment with Bemfola®- Gonal-f®, 29 from 249 patients with Bemfola® and 10 from 123 patients with Gonal-f® showed deviation in the protocol whereas, in case of Ovaleap® and Gonal-f®, 1patient from each group showed major protocol deviation. So, the final number of patients per protocol was 220 and 113 for Bemfola®-

Gonal-f® respectively while 152 and 145 for Ovaleap®- Gonal-f® respectively. Table 1 shows the summary of disposition of patients.

Table 1: Disposition of Patients.[34,35]

Enrolment (n)	**460[a]**		**398[b]**	
Screening Failures (n):	88[a]		99[b]	
Did not meet inclusion criteria	21		59	
Consent withdrawn	7		13	
Other reasons	60		27	
Randomized(intent-to-treat) (n)	**372[a]**		**299[b]**	
Assigned to	Bemfola®[a] (n=249)	Gonal-f®[a] (n=123)	Ovaleap®[b] (n=153)	Gonal-f®[b] (n=146)
Protocol deviation (n)	29	10	1	1
Not meeting inclusion criteria	7	2	--	--
Inaccurately completed assessment form	9	2	--	--
Termination during treatment	7	3	--	--
Endpoint not reached	1	2	--	--
Deviation after treatment	3	1	--	--
Treated not as randomized	2	--	--	--
Per protocol population(n)	220[a]	113[a]	152[b]	145[b]

[a]*Taken from reference*[34]

[b]*Taken from reference*[35]

d. Randomized clinical trials result

i. Bemfola® vs Gonal-f®

The baseline concentration of FSH (IU) was 6.9 for both. The duration of r-FSH medications and total mean dose (IU) was 10.6, 1555.7 vs 10.7, 1569.2. Lower peak Estradiol levels (8982.3 vs 7704.2 pmol/L) with a decline in OHSS incidence (14 vs 4). Though Bemfola® has higher chemical pregnancy rates (10.4% vs 4%), it non-significantly declined in live birth rate, clinical, and ongoing pregnancy (35.7%, 36.1%, 33.73% vs 43.9%, 44.7%, 41.46%) in comparison with Gonal-f®. The biochemical pregnancy was significantly comparable (46.58% vs 48.78%). Table 2 shows the summary of the entire data and Fig. 3 shows the graphical comparison of pregnancy results.

ii. Ovaleap® vs Gonal-f®

The baseline concentration of FSH (IU) was 7 and 7.3 respectively. The duration of r-FSH medications and total mean dose (IU) was 9.3, 1536 vs 9.7, 1614. Lower peak Estradiol levels (10070 vs 9534 pmol/L) with a decline in OHSS incidence (7 vs 4). Though Ovaleap® has higher chemical pregnancy rates (9.8% vs 5.4%), it non-significantly declined in live

birth rate, clinical, and ongoing pregnancy (26.7%, 28.1%, 27.4% vs 32.1%, 35.56%, 33.5%) in comparison with Gonal-f®. The biochemical pregnancy was significantly comparable (37.9% vs 41.1%). Table 2 shows the summary of the entire data and Fig. 3 shows the graphical comparison of pregnancy results.

Table 2: Bemfola® and Ovaleap® RCT data.[36,37]

Parameters	Bemfola®[a]	Gonal-f®[a]	Ovaleap®[b]	Gonal-f®[b]
FSH Baseline concentration (IU)	6.9	6.9	7	7.3
Days of FSH medication	10.6	10.7	9.3	9.7
Mean of total FSH (IU)	1555.7	1569.2	1536	1614
Estradiol (pmol/L)	8982.3	7704.2	10070	9534
OHSS(n)	14	4	7	4
Biochemical pregnancy (%)	46.58	48.78	37.9	41.1
Clinical pregnancy (%)	36.1	44.7	28.1	35.56
Ongoing pregnancy (%)	33.73	41.46	27.4	33.5
Live Birth Rate (%)	35.7	43.9	26.7[c]	32.1[c]
Chemical pregnancy (%)	10.4	4	9.8	5.4

[a] *Taken from reference[34]*

[b] *Taken from reference[35]*

[c] *Taken from reference[37]*

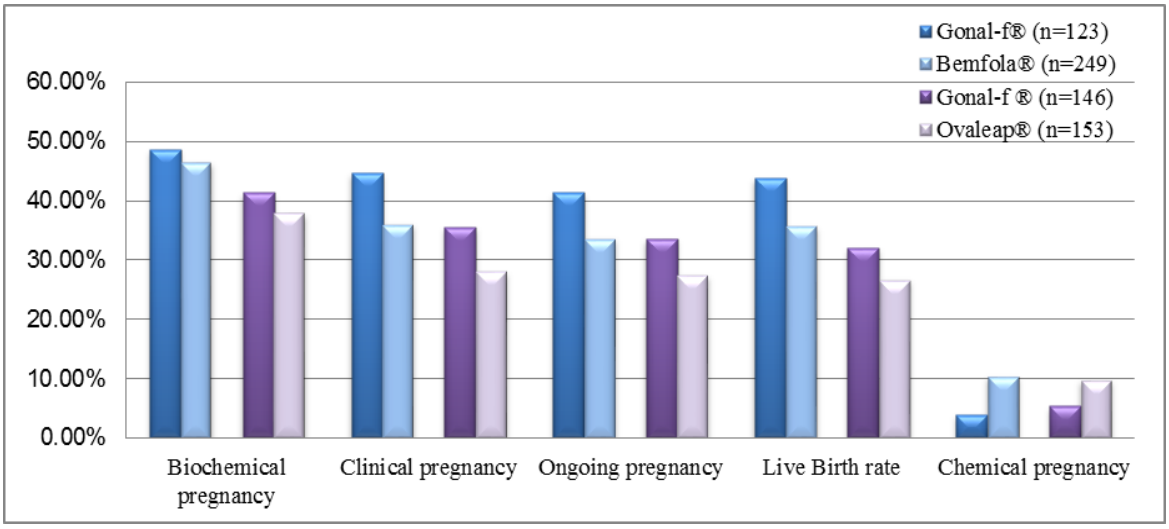

Fig. 3 Bemfola® and Ovaleap® RCT graphical representation.

6. CONCLUSION

Infertility has become a concerning factor in today's world due to many causative factors and the desire to have children has given rise to many modernized ways of treatment. One amongst them is biological and biosimilars. Biologicals have been found to be successful in the treatment of infertility. With the expiration of the patent of biologics, biosimilars have

emerged into the market and proven to be similar to its corresponding original biomolecule. The major advantage of biosimilars over the original biologics is its cost-effectiveness which provides a patient with economical therapy without compromising its safety, efficacy, and immunogenicity profile. Thus, the development of biosimilar r-hFSH products has given patients another treatment option increasing the availability and hopes for having a child.

Conflicts of interest

The authors have no conflicts of interest.

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